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EFFECTS OF DRY HEAT TREATMENT OF STYROBLOCK CONTAINERS ON COLONIZATION BY SELECTED FUNGI

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ABSTRACT

Tests were conducted to evaluate efficacy of dry heat (82.2°C for 10, 20, and 60 minutes) to reduce colonization by selected fungi within styroblock containers and residual seedling roots left in containers after seedling extraction. Dry heat alone was generally ineffective in significantly reducing fungal colonization. However, simply wetting container surfaces with water prior to treatment greatly improved efficacy. Fungi were readily killed both on container surfaces and within residual seedling roots when a thin film of water was present on containers prior to heat treatment. *Fusarium* species differed in their susceptibility to heat treatment. Wetting containers and exposure to dry heat is an effective alternative to hot water immersion for styroblock sanitization.

INTRODUCTION

Production of container-grown forest seedlings continues to increase in the inland Pacific Northwest. Seedlings are produced in containers that are normally reused for several crops. Styrofoam containers provide advantages over plastic or other type containers and have been adopted by most growers. However, they readily become colonized by fungi during seedling crop production and have to be effectively sanitized before new crops of seedlings are grown in them (James 1987, 1989; James et al. 1988). Several procedures have been used to try to effectively sanitize containers. Initially most nurseries treated their containers with high-pressure steam (James et al. 1988). Results were generally variable and other techniques included treatment with chemical surface sterilants such as bleach (aqueous sodium hypochlorite) and sodium metabisulfite (Dumroese et al. 1993; James and Sears 1990; James and Woollen 1989). Major drawbacks of chemical sterilants included worker exposure to and disposal of toxic chemicals (Dumroese et al. 1993).

Several years ago, many nursery growers began using hot water immersion treatments to sanitize containers (James 1992; James and Eggleston 1997; James and Woollen 1989; Peterson 1990, 1991; Sturrock and Dennis 1988). Although exposure times and water temperatures varied, in general, exposure of styroblock containers to 180oF (82.2oC) for at least five minutes effectively eliminated populations of potentially pathogenic fungi residing within containers. A serious drawback of hot water immersion is the high energy costs involved in keeping water hot for treatment of many containers (Peterson 1991). The process is also very labor intensive,

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requiring extensive handling of containers. This adds significantly to production costs. Therefore, we recently began to evaluate alternative treatments for sanitizing styroblock containers. Initially, we investigated use of radio frequency waves for heating container surfaces (James and Trent 2001). Such treatments were effective only if container surfaces were wetted prior to exposure. Water apparently effectively transmitted heat produced by the radio frequency waves to container surfaces where potential pathogens resided. Unfortunately, operational use of radio frequency waves is limited by the very high costs involved in obtaining the necessary treatment equipment (James and Trent 2001).

As part of our continuing efforts to develop cost-effective sanitation treatments for styroblock containers, we investigated use of dry heat in specially fabricated ovens. Results of these investigations are summarized here.

MATERIALS AND METHODS

Six styroblock containers used to grow several crops of seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho were tested. Each container was cut into thirds of approximately equal sizes. Within each third, 12 cells were randomly selected for sampling of selected fungi. The same cells were sampled before and after treatment. Two pieces of styrofoam opposite each other were aseptically removed from the bottom of each sampled cell and placed on an agar medium that is selective for *Fusarium* and closely related fungi (Komada 1975). At the same time, 10 pieces of residual roots attached to container surfaces were randomly selected from each third and aseptically placed on the selective medium. Plates with styrofoam pieces or roots were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7 days. Emerging fungi were identified to genus; selected isolates of *Fusarium* were transferred to potato dextrose and carnation leaf agar (Fisher et al. 1982) for species identification using the taxonomy of Nelson et al. 1983. Percentages of sampled cells colonized by particular fungi were calculated as "infection." Percentage of sampled styrofoam pieces (two per cell) were calculated as "colonization."

Five of the styroblock containers (designated A-E) were exposed to dry heat in an oven. Each third was exposed to 82.2°C for 10, 20 and 60 minutes, respectively. The sixth styroblock container (designated F) was first wetted thoroughly with warm tap water and then exposed to dry heat; each third was exposed to the same temperature – time regime as the other five containers. After treatment, containers were again sampled for presence of fungi. Results for containers A-E were collated and average infection and colonization means for particular fungal groups before and after treatment were compared statistically using the Kruskel-Wallis Test. Significant differences were assigned at $P \leq 0.05$ (Ott 1984). The same statistical tests were used to evaluate treatment effects on fungal infection and colonization for the wetted styroblock container (F).

RESULTS

Dry heat treatment did not significantly reduce infection by fungi, including those that are potentially pathogenic, regardless of exposure time (tables 1, 3, and 5). Such treatments were likewise ineffective in significantly reducing fungal colonization (tables 2, 4, and 6). However, exposure at 82.2°C for 60 min. resulted in significantly more samples not being colonized by any fungus (table 6), i.e., the heat was effective in killing some but not all fungal organisms within treated styroblock containers. Prolonged exposure to dry heat did not reduce colonization by potentially pathogenic organisms such as *Fusarium* spp.

On the other hand, with a few exceptions, wetting styroblock containers prior to heat treatment resulted in much greater fungal reductions. Even exposure for 10 minutes resulted in significantly reduced infection (table

1) and colonization (table 2) by potentially pathogenic as well as commonly saprophytic fungi. In many cases, all fungal organisms within sampled cells were killed when the containers had been wetted prior to heat treatment.

Tabulations of *Fusarium* species isolated from styroblock containers and residual roots as affected by dry heat treatments are summarized in tables 7 and 8, respectively. Generally, there were diverse populations of *Fusarium* on both container surfaces and colonizing residual roots. Some species appeared more heat sensitive than others. For example, on styroblock container surfaces, *F. avenaceum* (Fr.) Sacc. and *F. equiseti* (Corda) Sacc. were either eliminated or greatly reduced by heat treatment, whereas *F. proliferatum* (Matsushima) Nirenberg was detected at significantly higher levels following heat treatment (table 7). On residual seedling roots, significantly higher levels of *F. oxysporum* (Schlecht.), *F. solani* (Mart.) Appel & Wollenw. and *F. acuminatum* Ell. & Ev. were detected following heat treatments, whereas levels of *F. sporotrichioides* Sherb. and *F. sambucinum* Fuckel were significantly less following treatments (table 8).

Table 1. Effects of dry heat treatments (82.2°C – 10 minutes) on infection of styroblock containers by selected fungi.

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
A	58.3	41.7	16.7	0	58.3	91.7	0	0	91.7	66.7	0	0
B	8.3	41.7	0	0	25.0	8.3	8.3	16.7	100.0	100.0	0	0
C	25.0	58.3	0	0	58.3	75.0	8.3	8.3	83.3	50.0	0	0
D	16.7	25.0	0	0	8.3	8.3	91.7	58.3	91.7	91.7	0	0
E	0	0	0	0	75.0	75.0	66.7	66.7	25.0	75.0	0	0
Average[2]	21.7a	33.3a	3.3a	0a	45.0a	51.7a	35.0a	30.0a	78.3a	76.7a	0a	0a
F[3]	33.3a	0b	0a	0a	16.7a	8.3a	41.7a	8.3b	91.7a	0b	0a	100.0b

[1]Twelve cells sampled per block; the same cells were sampled before (pre) and after (post) heat treatment.
[2]Average of blocks A-E (all blocks underwent dry heat treatment); for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.
[3]Block was immersed in water prior to treatment; for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.

Table 2. Effects of dry heat treatments (82.2°C – 10 minutes) on colonization of styroblock containers by selected fungi.

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
A	29.2	25.0	8.3	0	37.5	66.7	0	0	70.8	54.2	0	0
B	4.2	25.0	0	0	16.7	4.2	4.2	8.3	91.7	95.8	0	0
C	12.5	33.3	0	0	33.3	54.2	4.2	4.2	66.7	37.5	0	0
D	8.3	12.5	0	0	4.2	4.2	62.5	29.2	83.3	75.0	0	0
E	0	0	0	0	50.0	50.0	41.7	54.2	16.7	62.5	0	0
Average[2]	10.8a	19.2a	1.7a	0a	28.3a	35.8a	22.5a	19.2a	65.8a	65.0a	0a	0a
F[3]	16.7a	0b	0a	0a	8.3a	4.2a	33.3a	4.2b	75.0a	0b	0a	87.5b

[1]Twelve cells sampled per block; the same cells were sampled before (pre) and after (post) heat treatment.
[2]Average of blocks A-E (all blocks underwent dry heat treatment); for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.
[3]Block was immersed in water prior to treatment; for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.

Table 3. Effects of dry heat treatments (82.2°C – 20 minutes) on infection of styroblock containers by selected fungi

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
A	33.3	16.7	0	0	91.7	100.0	0	16.7	66.7	58.3	0	0
B	25.0	41.7	0	0	33.3	33.3	8.3	8.3	100.0	83.3	0	0
C	0	8.3	0	0	75.0	75.0	16.7	8.3	91.7	75.0	0	0
D	8.3	0	0	0	25.0	16.7	100.0	8.3	75.0	100.0	0	0
E	0	0	0	0	100.0	83.3	66.7	83.3	33.3	25.0	0	0
Average[2]	13.3a	13.3a	0a	0a	65.0a	61.7a	73.3a	68.3a	38.3a	23.3a	0a	0a
F[3]	8.3a	8.3a	0a	0a	8.3a	0b	66.7a	0b	91.7a	25.0b	0a	100.0b

[1]Twelve cells sampled per block; the same cells were sampled before (pre) and after (post) heat treatment.
[2]Average of blocks A-E (all blocks underwent dry heat treatment); for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.
[3]Block was immersed in water prior to treatment; for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.

Table 4. Effects of dry heat treatments (82.2°C – 20 minutes) on colonization of styroblock containers by selected fungi.

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
A	16.7	8.4	0	0	66.7	83.3	0	8.3	41.7	33.3	0	0
B	12.5	29.2	0	0	16.7	16.7	4.2	4.2	87.5	70.8	0	0
C	0	4.2	0	0	50.0	50.0	8.3	0	79.2	45.8	0	0
D	4.2	0	0	0	12.5	8.3	87.5	4.2	50.0	95.3	0	0
E	0	0	0	0	62.5	70.8	41.7	50.0	16.7	16.7	0	0
Average[2]	6.7a	8.3a	0a	0a	41.7a	45.8a	28.3a	13.3a	55.0a	52.5a	0a	0a
F[3]	4.2a	4.2a	0a	0a	4.2a	0a	37.5a	0b	83.3a	12.5b	0a	87.5b

[1]Twelve cells sampled per block; the same cells were sampled before (pre) and after (post) heat treatment.
[2]Average of blocks A-E (all blocks underwent dry heat treatment); for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.
[3]Block was immersed in water prior to treatment; for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.

Table 5. Effects of dry heat treatments (82.2°C – 60 minutes) on infection of styroblock containers by selected fungi.

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
A	25.0	41.7	0	0	66.7	91.7	0	16.7	75.0	75.0	0	0
B	16.7	8.3	0	0	25.0	0	16.7	16.7	100.0	100.0	0	0
C	16.7	8.3	0	0	58.3	75.0	16.7	0	91.7	50.0	0	0
D	8.3	0	0	0	41.7	16.7	100.0	0	83.3	83.3	0	58.3
E	0	0	0	0	83.3	50.0	41.7	91.7	25.0	16.7	0	16.7
Average[2]	13.3a	11.7a	0a	0a	55.0a	48.3a	35.0a	25.0a	75.0a	65.0a	0a	25.0b
F[3]	25.0a	0b	0a	0a	8.3a	0a	41.7a	0b	91.7a	16.7b	0a	100.0b

[1]Twelve cells sampled per block; the same cells were sampled before (pre) and after (post) heat treatment.
[2]Average of blocks A-E (all blocks underwent dry heat treatment); for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.
[3]Block was immersed in water prior to treatment; for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.

Table 6. Effects of dry heat treatments (82.2°C – 60 minutes) on colonization of styroblock containers by selected fungi.

Block[1]	*Fusarium*		*Cylindrocarpon*		*Trichoderma*		*Penicillium*		Other Fungi		No Fungi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
A	12.5	25.0	0	0	41.7	79.2	0	8.3	58.3	62.5	0	0
B	8.3	4.2	0	0	12.5	0	8.3	8.3	95.8	91.7	0	0
C	8.3	4.2	0	0	33.3	54.2	8.3	0	75.0	29.2	0	25.0
D	4.2	0	0	0	20.8	8.3	91.7	0	66.7	58.3	0	33.3
E	0	0	0	0	75.0	25.0	25.0	70.8	16.7	12.5	0	8.3
Average[2]	6.7a	6.7a	0	0a	36.7a	33.3a	26.7a	17.5a	62.5a	50.8a	0a	13.3b
F[3]	12.5a	0b	0a	0a	4.2a	0a	29.2a	0b	79.2a	8.3b	0a	91.7b

[1]Twelve cells sampled per block; the same cells were sampled before (pre) and after (post) heat treatment.
[2]Average of blocks A-E (all blocks underwent dry heat treatment); for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.
[3]Block was immersed in water prior to treatment; for each fungus, average values followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis test.

Table 7. Effects of dry heat treatments on colonization of styroblock containers by different *Fusarium* species[1].

Fusarium species[2]		Block A	Block B	Block C	Block D	Block E	Block F	All Blocks[3]
FOXY	Pre	29	0	0	0	0	13	13.5a
	Post	7	23	0	0	0	0	8.7a
FSOL	Pre	14	0	0	0	0	0	5.4a
	Post	7	0	0	0	0	0	2.2a
FPRO	Pre	0	0	20	0	0	0	2.7a
	Post	21	15	75	17	0	0	32.6b
FAVE	Pre	0	17	0	50	0	0	8.1b
	Post	0	0	0	0	0	0	0a
FSPO	Pre	0	50	40	50	0	0	18.9a
	Post	0	23	17	0	0	0	10.9a
FACU	Pre	57	33	20	0	0	50	40.5a
	Post	65	31	8	83	0	100	43.4a
FEQU	Pre	0	0	20	0	0	37	10.9b
	Post	0	8	0	0	0	0	2.2a

[1]Values in table are percent of *Fusarium* isolates comprised of the appropriate species.
[2]*Fusarium* species: FOXY = *F. oxysporum*; FSOL = *F. solani*; FPRO = *F. proliferatum*; FAVE = *F. avenaceum*; FSPO = *F. sporotrichioides*; FACU = *F. acuminatum*; FEQU = *F. equiseti.*
[3]For each *Fusarium* species, means followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis Test.

Table 8. Effects of dry heat treatments on colonization of residual seedling roots by different *Fusarium* species[1].

Fusarium species[2]		Block A	Block B	Block C	Block D	Block E	Block F	All Blocks[3]
FOXY	Pre	0	0	0	0	0	0	0a
	Post	0	50	0	0	0	0	5.5b
FSOL	Pre	0	0	0	0	0	0	0a
	Post	25	0	0	0	0	0	5.5b
FPRO	Pre	89	22	83	0	0	0	48.4a
	Post	0	0	50	34	0	0	27.8a
FSPO	Pre	0	45	17	100	0	75	35.5b
	Post	0	50	12	0	0	0	11.2a
FACU	Pre	11	0	0	0	0	0	3.2a
	Post	75	0	38	66	0	100	50.0b
FSAM	Pre	0	33	0	0	0	25	12.9b
	Post	0	0	0	0	0	0	0a

[1]Values in table are percent of *Fusarium* isolates comprised of the appropriate species.
[2]*Fusarium* species: FOXY = *F. oxysporum*; FSOL = F. *solani*; FPRO = *F. proliferatum*; FSPO = *F. sporotrichioides;* FACU = *F. acuminatum*; FSAM = F. *sambucinum.*
[3]For *each Fusarium* species, means followed by the same letter are not significantly different (P=0.05) using the Kruskel-Wallis Test.

DISCUSSION

These tests confirmed the importance of water in conducting heat to kill adverse microorganisms within styroblock containers and colonizing residual seedling roots. Apparently, dry heat did not penetrate the styrofoam sufficiently to eliminate target microorganisms, although some organisms were reduced by exposure at 82.2°C for 60 min. The high temperature used in these tests was below the threshold causing some structural alterations of containers. Providing a thin layer of water on container surfaces was sufficient to conduct ambient heat to where microorganisms resided. It is unlikely that longer exposure periods, i.e., in excess of 60 min., would have greatly improved efficacy of dry heat treatments. However, following wetting, exposure of containers to less than 10 min. and perhaps even lower temperatures than were tested might have been effective.

Since water appears necessary to conduct external heat to locations where undesirable organisms reside on and within containers, the most practical concern involves the costs required for efficacious treatments. In the past, most nurseries have exposed containers to large amounts of heated water in immersion systems (James 1992; James and Eggleston 1987; James and Woollen 1989; Peterson 1990, 1991; Sturrock and Dennis 1988). Such treatments require large energy expenditures because water must be maintained at a high threshold temperatures in order to remain effective.

However, under such systems, very little of the water is in actual contact with container surfaces. Our work reported here and previously with radio frequency waves (James and Trent 2001) indicated that only a thin film of water is necessary to conduct ambient heat to where unwanted organisms reside. In other words, heating large amounts of water is not necessary and is more costly than heating equal volumes of air. Therefore, heating air can replace heating large volumes of water to obtain similar efficacy in sanitizing containers at lower energy costs. Of course, systems must be designed to reduce heat loss when replacing containers within ovens and containers surfaces will require wetting prior to heat exposure.

One question we have not addressed is the effects of either hot air or hot water immersion treatments on longevity and useful life span of styroblock containers. One treatment may be more damaging to containers than the other, resulting in another important "cost" of treatment.

Since *Fusarium* spp. are often the most important pathogens in container nurseries (James et al. 1991), we want mostly to reduce or eliminate these organisms in styroblock container treatments. As has been shown previously (James 1987, 1989, 1990, 2001; James et al. 1988; Sturrock and Dennis 1988), several different *Fusarium* spp. are common inhabitants of containers and provide a potential threat to future seedling crops if they are not either eliminated or greatly reduced. Some species are more important as potential pathogens than others. For example, we know that F. oxysporum and F. proliferatum are important pathogens under many nursery conditions (James et al. 1997, 2000). Also, certain isolates *of F. acuminatum, F. solani*, and *F. sporotrichioides* may also be aggressive pathogens in conifer nurseries (James 2000; James and Perez 1999, 2000). However, many isolates of all these species are primarily saprophytic or can induce disease only when infected hosts are severely stressed (James 2000; James and Perez 1999, 2000; James et al. 1991, 1997, 2000). Since we cannot easily differentiate pathogenic from nonpathogenic isolates (James et al. 1991), our goal has been to reduce or eliminate all *Fusarium* colonization within containers. That way we can be sure that pathogenic strains from containers cannot cause diseases on future seedling crops. Our work showed that some *Fusarium* species were more heat sensitive than others. However, in most tests, all isolates were killed when containers were wetted prior to exposure to dry heat. Perhaps low levels of *Fusarium* may survive following dry heat treatment of wetted containers, especially when fungi reside within residual seedling roots that have penetrated deep within the styrofoam. However, such low inoculum levels would not likely pose much of a potential threat to future seedling crops.

Our work indicated that dry heat can be as effective as hot water immersion treatments for sanitizing styroblock containers if the containers are wetted prior to treatment. Therefore, operational trials using this sanitizing technique are warranted. Such trials should evaluate treatment costs compared to existing hot water immersion methods and determine any effects on container integrity and longevity. Follow-up evaluation of treatment effects on subsequent seedling crops, i.e., disease production and seedling growth, would also be informative.

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